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Mary Hale, Information Branch Supervisor
308-4258, CM1-1E01

Voluntary Results Feedback Form

- *I am an examiner in Workgroup:* ☐ *Example: 1610*
- *Relevant prior art **found**, search results used as follows:*
  - ☐ 102 rejection
  - ☐ 103 rejection
  - ☐ Cited as being of interest.
  - ☐ Helped examiner better understand the invention.
  - ☐ Helped examiner better understand the state of the art in their technology.

Types of relevant prior art found:

- ☐ Foreign Patent(s)
- ☐ Non-Patent Literature
  (journal articles, conference proceedings, new product announcements etc.)

- *Relevant prior art **not found:***
  - ☐ Results verified the lack of relevant prior art (helped determine patentability).
  - ☐ Results were not useful in determining patentability or understanding the invention.

Comments:

Drop off or send completed forms to STIC/Biotech-Chem Library CM1 – Circ. Desk

Inventor Search

=> d ibib abs l22 1-1

L22 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:718000 HCAPLUS
DOCUMENT NUMBER: 127:356538
TITLE: construction of inactivation resistant factor VIII procoagulant and applications to hemophilia treatment
INVENTOR(S): **Kaufman, Randal J.; Pipe, Steven W. ; Amano, Kagehiro**
PATENT ASSIGNEE(S): Regents of the University of Michigan, USA; Kaufman, Randal J.; Pipe, Steven W.; Amano, Kagehiro
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740145	A1	19971030	WO 1997-US6563	19970424
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9732027	A1	19971112	AU 1997-32027	19970424
EP 910628	A1	19990428	EP 1997-927596	19970424
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000511407	T2	20000905	JP 1997-538216	19970424
US 2002132306	A1	20020919	US 2001-819098	20010411

PRIORITY APPLN. INFO.:
US 1996-16117P P 19960424
US 1996-17785P P 19960515
WO 1997-US6563 W 19970424

AB Novel purified and isolated nucleic acid sequences encoding procoagulant-active FVIII proteins are described. To det. whether specific amino acid sequences within FVIII A-domain inhibit secretion, chimeric proteins contg. the A1 and A2-domains of FVIII or FV were studied. The nucleic acid sequences of encode amino acid sequences corresponding to known human FVIII sequences where residue Phe309 is mutated. The nucleic acid sequences also encode human FVIII sequences where the APC cleavage sites, Arg336 and Ile562, are mutated. The nucleic acid sequences of sequences corresponding to known human FVIII sequences where the B-domain is deleted, the von Willebrand factor binding site is deleted, a thrombin cleavage site is mutated and an amino acid sequence spacer is inserted between the A2- and A3-domains. These nucleotide encode factor VIII proteins capable of secretion at levels higher than typically obtained with wild-type factor VIII. Methods of producing the FVIII proteins and pharmaceutical compns. contg. the nucleotide sequences or proteins as well as methods of treating patients suffering from hemophilia are also provided. A lower dosage of protein may be administered to the hemophiliac patient during FVIII replacement therapy. By utilizing the proteins described, the total exposure of protein to the patient is reduced, thereby lowering the likelihood of inhibitor formation.

```
=> d que stat l16
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                 ROTEIN?
L2            49 SEA FILE=HCAPLUS ABB=ON  ?FVIII?(W)?PROTEIN?
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L5             1 SEA FILE=HCAPLUS ABB=ON  L4 AND (?PHE309? OR PHE?(W)309?)
L6             1 SEA FILE=HCAPLUS ABB=ON  L1 OR L5
L7             2 SEA FILE=HCAPLUS ABB=ON  L2 AND (?PHE309? OR PHE?(W)309?)
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L9            56 SEA FILE=HCAPLUS ABB=ON  ?FVIII?(W)(?PROTEIN? OR ?POLYPEPTIDE?)
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L13            2 SEA FILE=HCAPLUS ABB=ON  L9 AND (?PHE309? OR PHE?(W)309?)
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L15            8 SEA FILE=HCAPLUS ABB=ON  L11 AND ?MUTAT?
L16            9 SEA FILE=HCAPLUS ABB=ON  L14 OR L15

=> d ibib abs l16 1-9

L16  ANSWER 1 OF 9  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2002:450685  HCAPLUS
DOCUMENT NUMBER:          137:260728
TITLE:                    Inhibitor development in correlation to factor VIII
                          genotypes
AUTHOR(S):                Oldenburg, J.; El-Maarri, O.; Schwaab, R.
CORPORATE SOURCE:         Institute of Transfusion Medicine and Immune
                          Haematology of the DRK Blood Donor Service Hessen,
                          Frankfurt, 60528, Germany
SOURCE:                   Haemophilia (2002), 8(Suppl. 2), 23-29
                          CODEN: HAEMF4; ISSN: 1351-8216
PUBLISHER:                Blackwell Science Ltd.
DOCUMENT TYPE:            Journal; General Review
LANGUAGE:                 English
AB   A review. Alloantibodies (inhibitors) against factor VIII (FVIII) develop
     in 20-30% of patients with severe hemophilia A and render classical FVIII
     substitution therapy ineffective. Several studies have shown that genetic
     factors, the type of FVIII gene mutation and immune response
     genes (e.g. the Major Histocompatibility Complexes), influence the risk of
     inhibitor formation. In particular, the type of FVIII gene
     mutation has proven to be a decisive risk factor. Patients with
     severe mol. gene defects (e.g. large deletions, nonsense mutations
     , intron-22 inversion) and no endogenous FVIII synthesis have a 7-10 times
     higher inhibitor prevalence than patients with milder mol. gene defects
     (e.g. missense mutations, small deletions, splice site
     mutations). To date, at least 10 distinct classes of
     mutations have been shown which have differing risks of assocd.
     inhibitor formation. A challenging observation in inhibitor patients is
     the heterogeneity of the antibody epitopes with respect to their no. and
     their specificity. At least five epitopes in the FVIII mol. have been
     identified that constitute the targets for antibodies in most inhibitor
     patients. These epitopes are located in the ar3 region and the A2, A3,
     C1, C2 domains which correspond to the functional binding sites of the
     ligands of the FVIII protein. At present, the
     determinants of the characteristics of these epitopes and the subsequent
     inhibitor titer are unknown. A relationship of the mutation
     site and the epitope localization has been shown for some individual
     patients with mild hemophilia A. However, in severely affected hemophilia
     A patients, the influence of patient genetics on inhibitor titer and
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epitope specificity has yet to be elucidated.
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:30754 HCAPLUS
DOCUMENT NUMBER: 135:3952
TITLE: Genotype-phenotype correlation in hemophilia A
AUTHOR(S): Graw, J.; Brackmann, H.-H.; Oldenburg, J.; Schramm, W.; Schwaab, R.
CORPORATE SOURCE: Inst. Saugetiergenet., GSF-Forschungszentrum f. Umwelt und Gesundheit GmbH, Oberschleissheim, Germany
SOURCE: Hemophilia Symposium, 30th, Hamburg, Germany, 1999 (2001), Meeting Date 1999, 13-21. Editor(s): Scharrer, Inge; Schramm, Wolfgang. Springer-Verlag: Berlin, Germany.
CODEN: 69AUSD
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Within the second phase of the German **Human** Genome Project, a series of investigations will be founded concerning a systematic genotype-phenotype correlation in hemophilia A in Germany. In particular, the hemophilia A phenotype will be correlated to the type of **mutations**, the affected domains in the factor VIII protein, and the prodn. of alloantibodies. The identification of the genotype of all severe cases of hemophilia A in Germany is expected within the next 3 yr and this will be compared with the individual treatment protocols. During this study, domains in the **FVIII protein** will be identified, which are mainly responsible for antibody prodn. The expected results of the proposed study open the way for new strategies in the therapy of hemophilia A. The project makes important contributions to the understanding of the efficacy of pharmaceutical active substances at genetically distinct targets. During the project, a large no. of disease-assocd. alleles will be identified to avoid adverse reactions during therapy. This information is important for companies producing recombinant FVIII concs. to allow an optimized design for novel recombinant factor VIII products.
REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:34104 HCAPLUS
DOCUMENT NUMBER: 128:139327
TITLE: The molecular basis for cross-reacting material-positive hemophilia A due to missense **mutations** within the A2-domain of factor VIII
AUTHOR(S): Amano, Kagehiro; Sarkar, Rita; Pemberton, Susan; Kemball-Cook, Geoffrey; Kazazian, Haig H., Jr.; Kaufman, Randal J.
CORPORATE SOURCE: The Howard Hughes Medical Institute and the Department of Biological Chemistry and University of Michigan Medical Center, Ann Arbor, MI, 48109-0650, USA
SOURCE: Blood (1998), 91(2), 538-548
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Factor VIII (FVIII) is the protein defective in the bleeding disorder hemophilia A. Approx. 5% of hemophilia A patients have normal amts. of a dysfunctional **FVIII protein** and are termed

cross-reacting material (CRM)-pos. The majority of genetic alterations that result in CRM-pos. hemophilia A are missense **mutations** within the A2-domain. To det. the mechanistic basis of the genetic defects within the A2-domain for FVIII function the authors constructed six **mutations** within the FVIII cDNA that were previously found in five CRM-pos. hemophilia A patients (R527W, S558F, I566T, V634A, and V634M) and one CRM-reduced hemophilia A patient (DeltaF652/3). The specific activity for each mutant secreted into the conditioned medium from transiently transfected COS-1 cells correlated with published data for the patient's plasma-derived FVIII, confirming the basis of the genetic defect. SDS-PAGE anal. of immunopptd. **FVIII protein** radiolabeled in COS-1 cells showed that all CRM-pos. mutant proteins were synthesized and secreted into the medium at rates similar to wild-type FVIII. The majority of the DeltaF652/3 mutant was defective in secretion and was degraded within the cell. All mutant **FVIII proteins** were susceptible to thrombin cleavage, and the A2-domain fragment from the I566T mutant had a reduced mobility because of use of an introduced potential N-linked glycosylation site that was confirmed by N-glycanase digestion. To evaluate interaction of FVIII with factor IXa, the authors performed an inhibition assay using a synthetic peptide corresponding to FVIII residues 558 to 565, previously shown to be a factor IXa interaction site. The concn. of peptide required for 50% inhibition of FVIII activity (IC50) was reduced for the I566T (800 .mu.mol/L) and the S558F (960 .mu.mol/L) mutants compared with wild-type FVIII (>2,000 .mu.mol/L). N-glycanase digestion increased I566T mutant FVIII activity and increased its IC50 for the peptide (1,400 .mu.mol/L). In comparison to S558F, a more conservative mutant (S558A) had a sixfold increased specific activity that also correlated with an increased IC50 for the peptide. These results provided support that the defects in the I566T and S558F FVIII mols. are caused by steric hindrance for interaction with factor IXa.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:718000 HCAPLUS
DOCUMENT NUMBER: 127:356538
TITLE: construction of inactivation resistant factor VIII **procoagulant** and applications to hemophilia treatment
INVENTOR(S): Kaufman, Randal J.; Pipe, Steven W.; Amano, Kagehiro
PATENT ASSIGNEE(S): Regents of the University of Michigan, USA; Kaufman, Randal J.; Pipe, Steven W.; Amano, Kagehiro
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740145	A1	19971030	WO 1997-US6563	19970424

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN,

ML, MR, NE, SN, TD, TG

AU 9732027	A1	19971112	AU 1997-32027	19970424
EP 910628	A1	19990428	EP 1997-927596	19970424
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000511407	T2	20000905	JP 1997-538216	19970424
US 2002132306	A1	20020919	US 2001-819098	20010411
PRIORITY APPLN. INFO.:			US 1996-16117P P	19960424
			US 1996-17785P P	19960515
			WO 1997-US6563 W	19970424

AB Novel purified and isolated nucleic acid sequences encoding **procoagulant-active FVIII proteins** are described. To det. whether specific amino acid sequences within FVIII A-domain inhibit secretion, chimeric proteins contg. the A1 and A2-domains of FVIII or FV were studied. The nucleic acid sequences of encode amino acid sequences corresponding to known **human** FVIII sequences where residue **Phe309** is **mutated**. The nucleic acid sequences also encode **human** FVIII sequences where the APC cleavage sites, Arg336 and Ile562, are **mutated**. The nucleic acid sequences of sequences corresponding to known **human** FVIII sequences where the B-domain is deleted, the von Willebrand factor binding site is deleted, a thrombin cleavage site is **mutated** and an amino acid sequence spacer is inserted between the A2- and A3-domains. These nucleotide encode factor VIII proteins capable of secretion at levels higher than typically obtained with wild-type factor VIII. Methods of producing the **FVIII proteins** and pharmaceutical compns. contg. the nucleotide sequences or proteins as well as methods of treating patients suffering from hemophilia are also provided. A lower dosage of protein may be administered to the hemophiliac patient during FVIII replacement therapy. By utilizing the proteins described, the total exposure of protein to the patient is reduced, thereby lowering the likelihood of inhibitor formation.

L16 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER:	1997:652481 HCAPLUS
DOCUMENT NUMBER:	127:329321
TITLE:	Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation factor VIII
AUTHOR(S):	Swaroop, Manju; Moussalli, Micheline; Pipe, Steven W.; Kaufman, Randal J.
CORPORATE SOURCE:	Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI, 48109, USA
SOURCE:	Journal of Biological Chemistry (1997), 272(39), 24121-24124 CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER:	American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Coagulation factor VIII (FVIII) and factor V are homologous glycoproteins that have a domain structure of A1-A2-B-A3-C1-C2. FVIII is a heterodimer of the heavy chain (domains A1-A2-B) and the light chain (domains A3-C1-C2) in a metal ion-dependent assocn. between the A1- and A3-domains. Previous studies identified a 110-amino acid region within the FVIII A-domain that inhibits its secretion and contains multiple short peptide sequences that have potential to bind Ig-binding protein (BiP). FVIII secretion requires high levels of intracellular ATP, consistent with an ATP-dependent release from BiP. Site-directed mutagenesis was used to elucidate the importance of the potential BiP-binding sites in FVIII

secretion. Mutation of Phe at position 309 to Ser or Ala enhanced the secretion of functional FVIII and reduced its ATP dependence. The F309S FVIII had a specific activity, thrombin activation profile, and heat inactivation properties similar to those of wild-type FVIII. However, F309S FVIII displayed increased sensitivity to EDTA-mediated inactivation that is known to occur through metal ion chelation-induced dissocn. of the heavy and light chains of FVIII. The results support that **Phe309** is important in high affinity heavy and light chain interaction, and this correlates with a high affinity BiP-binding site. Introduction of the F309S mutation into other secretion defective FVIII mutants rescued their secretion, demonstrating the ability of the this mutation to improve secretion of mutant **FVIII proteins** retained in the cell.

L16 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:137032 HCAPLUS
DOCUMENT NUMBER: 126:184435
TITLE: Molecular genetics of hemophilia A
AUTHOR(S): De Brasi, Carlos D.; Slavutsky, Irma R.; Larripa, Irene B.
CORPORATE SOURCE: Dep. Genet., Acad. Nacional Med., Buenos Aires, Argent.
SOURCE: Medicina (Buenos Aires) (1996), 56(5/1), 509-517
CODEN: MEDCAD; ISSN: 0025-7680
PUBLISHER: Sociedad Argentina de Investigacion Clinica
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Spanish

AB A review with 32 refs. Hemophilia A (HemA), an X-linked genetic disease, is the most common **coagulation** disorder with an incidence of about 1-2 in 10,000 males and is caused by **mutations** in the factor VIII (FVIII) **coagulation** gene. Firstly, some clin. aspects of the HemA are presented: the current methods to assess both the amt. and activity of FVIII, the severity range obsd., and the presence of inhibitor antibodies against the therapeutic FVIII. A discussion of the relation of the structural domains of the **FVIII protein** and of the amino acid sequence and their functions follows. An activation-inactivation model of the successive peptide bonds cleavages of the FVIII is also presented. After the cloning of the FVIII gene in 1984, almost all types of HemA causing **mutations** have been characterized. However, the size and complexity of this gene prevented a screening of the full range of **mutations** for an accurate mol. diagnosis. Moreover, most of the patients with moderate and mild disease have missense **mutations**, whereas approx. half of severe patients have nonsense, frameshift, and some missense **mutations**. There are also less frequently **mutations** such as deletions and insertions leading to severe phenotype and **mutations** affecting mRNA splicing and duplications causing both severe and mild HemA. In genetic counseling of HemA families, studies at the DNA level using intragenic and/or extragenic polymorphism anal. have been used. But this approach is not entirely satisfactory because it fails in several situations. Most of the causing **mutations** described above are private, and they have been found in only a few unrelated families. Recently, a common mol. inversion of the FVIII gene was identified in 50% of unrelated patients with severe HemA. The inversion is mediated by the presence of three copies of a particular DNA sequence (termed F8A gene). One copy is located within intron 22 of the FVIII gene and the other two, 500 kb upstream. An homologous recombination mechanism was proposed for the inversion between an intragenic copy of the F8A gene and either the distal (80% of the inversion) or the proximal copy (20%). Both of these inversions lead to severe HemA because no intact FVIII is produced and can

be easily diagnosed by Southern blot anal. This inversion originates almost exclusively in male germ cells, because pairing Xq with its homolog in female meiosis would probably inhibit the proposed intrachromosome recombination. The mol. anal. of the inversion of intron 22 is now considered as the first line for families with severe HemA patients. In recent years, the treatment of patients with hemophilia A and B has been i.v. injection of FVIII or FIX concs., resp. This regimen of regular injection of plasmatic proteins bears a high risk of infection by contaminating viruses (HIV, HBV, etc.). Future treatment for patients with hemophilia may include the use of either gene therapy or recombinant **coagulation** factors. Both strategies would completely avoid the infection risk offering a safe and effective treatment for the disease. Recombinant factors, obtained by genetic engineering methods, provide a renewable and unlimited source of FVIII or FIX. The clin. trials of recombinant factors have already started in mid-1995 giving pos. results. Gene therapy for hemophilia is now in the pre-clin. stage but offers the prospect of a cure for the disease, thus potentially freeing patients from regular injections of the lacking protein. However, expts. in animal models suggest that it may be difficult to obtain adequate therapeutic levels of factors for long periods of time. Recently, a retroviral-mediated gene delivery of **human** FVIII in mice has been reported using the ex vivo strategy of gene therapy. Therapeutic levels of FVIII in the circulation were obtained for >1 wk and it was also obsd. that the capacity of primary cells to deliver FVIII in blood was strongly dependent on the site of implantation. Although much work remains to be done, these pos. results are encouraging for the future of gene therapy for this relatively common genetic disease.

L16 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:469427 HCAPLUS
DOCUMENT NUMBER: 119:69427
TITLE: Spectrum of **mutations** in CRM-positive and CRM-reduced hemophilia A
AUTHOR(S): McGinniss, Matthew J.; Kazazian, Haig H., Jr.; Hoyer, Leon W.; Bi, Lei; Inaba, Hiroshi; Antonarakis, Stylianos E.
CORPORATE SOURCE: Sch. Med., Johns Hopkins Univ., Baltimore, MD, USA
SOURCE: Genomics (1993), 15(2), 392-8
CODEN: GNMCEP; ISSN: 0888-7543
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hemophilia A is due to the functional deficiency of factor VIII (FVIII, gene locus F8C). Although half the patients have no detectable **FVIII protein** in their plasma, the more rare patients (.apprx.5%) have normal levels of a dysfunctional FVIII and are termed cross-reacting material (CRM)-pos. More commonly (.apprx.45%), patients have plasma **FVIII protein** reduced to an extent roughly comparable to the level of FVIII activity and are designated CRM-reduced. The authors used denaturing gradient gel electrophoresis to screen for **mutations** within F8C gene of 11 patients (6 CRM-pos., 5 CRM-reduced) and identified 9 different **mutations** in 9 patients after analyses of all 26 exons, the promoter region, and the polyadenlyation site. Six **mutations** have not been described previously. Five were missense (Ser289Leu, Ser558Phe, Val634Ala, Val634Met, Asn1441Lys), and the sixth was a 3-bp deletion (.DELTA.Phe652). A review of the literature and the assay of FVIII antigen in 5 hemophilia A patients with previously identified missense **mutations** from this lab. yielded a total of 20 other unique CRM-reduced and CRM-pos. **mutations**. Almost all CRM-pos./reduced **mutations** (24/26) were missense, and many (12/26) occurred at CpG dinucleotides.

The authors examd. 19 missense **mutations** for evolutionary conservation using the portions of the porcine and murine F8C sequences that are known, and 18/19 amino acid residues altered by **mutation** in these patients were conserved. Almost 50% of **mutations** (11/26) clustered in the A2 domain, suggesting that this region is crit. for the function of FVIII. The results indicate a nonrandom distribution of **mutations** and suggest that **mutations** in a limited no. of FVIII regions may cause CRM-pos. and CRM-reduced hemophilia A.

L16 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:133616 HCAPLUS
DOCUMENT NUMBER: 112:133616
TITLE: Recurrent **mutations** and three novel rearrangements in the factor VIII gene of hemophilia A patients of Italian descent
AUTHOR(S): Casula, L.; Murru, S.; Pecorara, M.; Ristaldi, M. S.; Restagno, G.; Mancuso, G.; Morfini, M.; De Biasi, R.; Baudo, F.; et al.
CORPORATE SOURCE: Ist. Clin. Biol., Univ. Stud. Cagliari, Cagliari, Italy
SOURCE: Blood (1990), 75(3), 662-70
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Six different **mutations** are described in the factor VIII (FVIII) gene detected by DNA anal. of 100 hemophilia A (HA) patients of Italian descent. In two of them, with a severe clin. picture, two novel deletions were identified, one in the middle of the FVIII gene from exons 7 to 22 and the other encompassing the entire factor VIII gene. Both of these patients produced antibodies to factor VIII. In a patient with mild HA a duplication of exon 13 was found, which is a rearrangement not yet described within the FVIII gene. A possible explanation for the mild phenotype in this patient is that the mol. defect results in the prodn. of an unstable **FVIII protein** with residual 10% FVIII activity. Screening by TaqI restriction endonuclease detected three **mutations** that were further characterized by direct sequencing on amplified DNA: a C-T substitution at codon 1960, in exon 18, converting the codon for arginine to a nonsense codon; and a G-A substitution at codon 2228 and 2326, in exons 24 and 26 resp., resulting in the substitution of glutamine for arginine. All three of these **mutations** have been previously described. The nonsense **mutation** and the codon 2228 G-A **mutation** was found in patients with severe HA, while the codon 2326 G-A **mutation** was assocd. with a quite severe condition. These results confirm that the mol. bases of HA are very heterogeneous and provide further evidence that recurrent **mutations** are not uncommon in this system.

L16 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:5568 HCAPLUS
DOCUMENT NUMBER: 112:5568
TITLE: An arginine to cysteine amino acid substitution at a critical thrombin cleavage site in a dysfunctional factor VIII molecule
AUTHOR(S): Shima, Midori; Ware, Jerry; Yoshioka, Akira; Fukui, Hiromu; Fulcher, Carol A.
CORPORATE SOURCE: Scripps Clin. Res. Found., La Jolla, CA, USA
SOURCE: Blood (1989), 74(5), 1612-17
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The factor VIII (**FVIII**) **protein** and the nucleotide sequence were analyzed around 2 thrombin cleavage sites, at arginine 372 in the FVIII heavy chain and arginine 1689 in the FVIII light chain in a naturally occurring dysfunctional FVIII variant, FVIII Okayama. The patient was a 42-yr-old hemophiliac with a FVIII **coagulant** activity of 0.03 U/mL and a FVIII antigen level of 0.8 U/mL. The patient's FVIII was not thrombin activatable to levels seen in normal plasma. Immunoblotting of partially purified FVIII Okayama and normal FVIII showed that thrombin cleavage of the 92 kilodalton (Kd) heavy chain was impaired in the mutant protein. The patient's genomic DNA was amplified using the polymerase chain reaction with 2 sets of synthetic oligonucleotide primers spanning amino acid residues 319-400 and 1630-1720. Sequence anal. of the amplified DNA fragments revealed a cytosine to thymine transition, converting an arginine to a cysteine codon at residue 372. No abnormality was found in the FVIII light chain region analyzed. The patient's hemophilic brother and carrier mother revealed the same **mutation**. Thus, the pathogenesis of hemophilia A in this patient is probably due to an arginine to cysteine substitution at a thrombin cleavage site in the FVIII heavy chain.

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=> d que stat l18
L1             1 SEA FILE=HCAPLUS ABB=ON  ?COAGULANT?(W)?ACTIVE?(3A)?FVIII?(W)?P
                 ROTEIN?
L2            49 SEA FILE=HCAPLUS ABB=ON  ?FVIII?(W)?PROTEIN?
L3            36 SEA FILE=HCAPLUS ABB=ON  L2 AND ?COAG?
L4            27 SEA FILE=HCAPLUS ABB=ON  L3 AND ?HUMAN?
L5             1 SEA FILE=HCAPLUS ABB=ON  L4 AND (?PHE309? OR PHE?(W)309?)
L6             1 SEA FILE=HCAPLUS ABB=ON  L1 OR L5
L7             2 SEA FILE=HCAPLUS ABB=ON  L2 AND (?PHE309? OR PHE?(W)309?)
L8             2 SEA FILE=HCAPLUS ABB=ON  L6 OR L7
L9            56 SEA FILE=HCAPLUS ABB=ON  ?FVIII?(W)(?PROTEIN? OR ?POLYPEPTIDE?)
L10           42 SEA FILE=HCAPLUS ABB=ON  L9 AND ?COAG?
L11           33 SEA FILE=HCAPLUS ABB=ON  L10 AND ?HUMAN?
L13            2 SEA FILE=HCAPLUS ABB=ON  L9 AND (?PHE309? OR PHE?(W)309?)
L14            2 SEA FILE=HCAPLUS ABB=ON  L8 OR L13
L15            8 SEA FILE=HCAPLUS ABB=ON  L11 AND ?MUTAT?
L16            9 SEA FILE=HCAPLUS ABB=ON  L14 OR L15
L17           18 SEA L16
L18            8 DUP REMOV L17 (10 DUPLICATES REMOVED)

=> d ibib abs l18 2-8

L18  ANSWER 1 OF 8  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER:   2002:434078  BIOSIS
DOCUMENT NUMBER:    PREV200200434078
TITLE:              Expression of factor VIII in recombinant and transgenic
                    systems.
AUTHOR(S):          Soukharev, Serguei; Hammond, David; Ananyeva, Natalya M.;
                    Anderson, Julia A. M.; Hauser, Charlotte A. E.; Pipe,
                    Steven; Saenko, Evgueni L. (1)
CORPORATE SOURCE:   (1) Department of Biochemistry, Holland Laboratory,
                    American Red Cross, 15601 Crabbs Branch Way, Rockville, MD,
                    20855 USA
SOURCE:             Blood Cells Molecules and Diseases, (March April, 2002)
                    Vol. 28, No. 2, pp. 234-248. http://www.academicpress.com/b
                    cmd. print.
                    ISSN: 1079-9796.
DOCUMENT TYPE:      General Review
LANGUAGE:           English
AB   Deficiency in a coagulation factor VIII (FVIII) causes a genetic
     disorder hemophilia A, which is treated by repeated infusions of expensive
     FVIII products. Recombinant FVIII (rFVIII), the culmination of years of
     extensive international research, is an important alternative to
     plasma-derived FVIII (pdFVIII) and is considered to have a higher margin
     of safety. Advances in biotechnology allowed production of rFVIII at
     industrial scale, which significantly improved treatment of hemophilia A
     patients. We review the contemporary methods used for FVIII expression in
     mammalian cell culture systems and discuss the factors responsible for
     insufficient recoveries of rFVIII, such as inefficient accumulation of
     FVIII mRNA in the cell, complexity of the mechanisms of FVIII secretion,
     and instability of secreted FVIII. The approaches to improve the yield of
     rFVIII in cell culture systems include genetic engineering of
     B-domain-deleted FVIII, introduction of introns into FVIII cDNA constructs
     for more efficient processing and accumulation of FVIII mRNA, and
     introduction of mutations into chaperone-binding sites of FVIII
     to improve its secretion. Design of FVIII with prolonged half-life in vivo
     is considered as another promising direction in improving rFVIII
     protein and efficiency of hemophilia A therapy. As an alternative
     to expression of rFVIII in cell culture systems, we discuss production of
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rFVIII in transgenic animals, where high levels of rFVIII have been successfully secreted into milk. We also pay attention to the major limitations of this approach, such as safety issues associated with potential transmission of animal pathogens. Finally, we present a brief characterization of commercial recombinant FVIII products currently available on the market for hemophilia A treatment.

L18 ANSWER 2 OF 8 JICST-EPlus COPYRIGHT 2003 JST
ACCESSION NUMBER: 1000752153 JICST-EPlus
TITLE: Identification of Plasma Antibody Epitopes and Gene Abnormalities in Japanese Hemophilia A Patients with Factor VIII Inhibitor.
AUTHOR: SUGIHARA T
TAKAHASHI I; KAMIYA T
KOJIMA T; OKAMOTO Y; YAMAMOTO K; MATSUSHITA T; SAITO H
CORPORATE SOURCE: Hekinan City Hospital
Japanese Red Cross Aichi Blood Center
Nagoya Univ. School Of Medicine
SOURCE: Nagoya J Med Sci, (2000) vol. 63, no. 1/2, pp. 25-39.
Journal Code: G0722A (Fig. 3, Tbl. 3, Ref. 37)
CODEN: NJMSAG; ISSN: 0027-7622
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: English
STATUS: New
AB Eleven Japanese hemophilia A patients with anti-factor VIII (FVIII) inhibitors were studied to localize both their inhibitory antibody epitopes and their genotypes. The inhibitor epitopes were studied in nine severe hemophilia A patients by means of a scanning method using the oligopeptide panel covering the **FVIII polypeptides** without the B domain. The 107 15 mer-peptides were synthesized on solid-phase pins and analyzed for their reactivity with diluted patient plasma. As indicated previously, a series of peptides corresponding to the A2 and C2 domains were recognized by plasma antibodies from 2 patients and 4 patients, respectively. In contrast, all the antibodies bound to several epitopes in the A3 domain, while an epitope 1809-1821 covering the putative factor IX binding site was found in 3 patients. Southern blotting analysis showed that 8 out of 11 patients had either gene deletions or inversions of the FVIII gene, indicating a higher proportion of gross gene alterations in inhibitor-positive hemophilia A patients. However, the correlation of gene abnormality type with epitope location was not fully established. (author abst.)

L18 ANSWER 3 OF 8 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999081615 MEDLINE
DOCUMENT NUMBER: 99081615 PubMed ID: 9864159
TITLE: Mild hemophilia A caused by increased rate of factor VIII A2 subunit dissociation: evidence for nonproteolytic inactivation of factor VIIIa in vivo.
AUTHOR: Pipe S W; Eickhorst A N; McKinley S H; Saenko E L; Kaufman R J
CORPORATE SOURCE: Departments of Pediatrics and Biological Chemistry, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI, 48109-0650, USA.
CONTRACT NUMBER: HD28820 (NICHD)
HL52173 (NHLBI)
SOURCE: BLOOD, (1999 Jan 1) 93 (1) 176-83.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216
Entered Medline: 19990204

AB Approximately 5% of hemophilia A patients have normal amounts of a dysfunctional factor VIII (**FVIII**) **protein** and are termed cross-reacting material (CRM)-positive. FVIII is a heterodimer (domain structure A1-A2-B/A3-C1-C2) that requires thrombin cleavage to elicit **procoagulant** activity. Thrombin-activated FVIII is a heterotrimer with the A2 subunit (amino acid residues 373 to 740) in a weak ionic interaction with the A1 and A3-C1-C2 subunits. Dissociation of the A2 subunit correlates with inactivation of FVIII. Recently, a phenotype of CRM-positive hemophilia A patients has been characterized whose plasma displays a discrepancy between their FVIII activities, where the one-stage clotting assay displays greater activity than the two-stage clotting assay. One example is a missense **mutation** where ARG531 has been substituted by HIS531. An FVIII cDNA construct was prepared containing the ARG531(HIS) **mutation** and the protein was expressed in COS-1 monkey cells by transient DNA transfection. Metabolic labeling with [35S]-methionine demonstrated that ARG531(HIS) was synthesized at an equal rate compared with FVIII wild-type (WT) but had slightly reduced antigen in the conditioned medium, suggesting a modest secretion defect. A time course of structural cleavage of ARG531(HIS) demonstrated identical thrombin cleavage sites and rates of proteolysis as FVIII WT. Similar to the patient phenotypes, ARG531(HIS) had discrepant activity as measured by a one-stage activated partial thromboplastin time (aPTT) clotting assay (36% +/- 9.6% of FVIII WT) and a variation of the two-stage assay using a chromogenic substrate (COAMATIC; 19% +/- 6.9% of FVIII WT). Partially purified FVIII WT and ARG531(HIS) proteins were subjected to functional activation by incubation with thrombin. ARG531(HIS) demonstrated significantly reduced peak activity and was completely inactivated after 30 seconds, whereas FVIII WT retained activity until 2.5 minutes after activation. Because the ARG531(HIS) missense **mutation** predicts a charge change to the A2 subunit, we hypothesized that the ARG531(HIS) A2 subunit could be subject to more rapid dissociation from the heterotrimer. The rate of A2 dissociation, using an optical biosensor, was determined to be fourfold faster for ARG531(HIS) compared with FVIII WT. Because the two-stage assay involves a preincubation phase before assay measurement, an increased rate of A2 dissociation would result in an increased rate of inactivation and reduced specific activity.

L18 ANSWER 4 OF 8 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 1997-535830 [49] WPIDS
DOC. NO. CPI: C1997-171386
TITLE: Modified **human** pro-**coagulant** active factor VIII - can be administered to haemophiliacs, i.e. factor VIII replacement therapy.
DERWENT CLASS: B04 D16
INVENTOR(S): AMANO, K; KAUFMAN, R J; PIPE, S W
PATENT ASSIGNEE(S): (UNMI) UNIV MICHIGAN; (AMAN-I) AMANO K; (KAUF-I) KAUFMAN R J; (PIPE-I) PIPE S W
COUNTRY COUNT: 77
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9740145	A1	19971030	(199749)*	EN	57

RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN YU
AU 9732027 A 19971112 (199811)
EP 910628 A1 19990428 (199921) EN
R: AL AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV MC NL PT RO SE SI
JP 2000511407 W 20000905 (200047) 60
US 2002132306 A1 20020919 (200264)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9740145	A1		WO 1997-US6563	19970424
AU 9732027	A		AU 1997-32027	19970424
EP 910628	A1		EP 1997-927596	19970424
			WO 1997-US6563	19970424
JP 2000511407	W		JP 1997-538216	19970424
			WO 1997-US6563	19970424
US 2002132306	A1	Provisional	US 1996-16117P	19960424
		Provisional	US 1996-17785P	19960515
			US 2001-819098	20010411

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9732027	A	Based on	WO 9740145
EP 910628	A1	Based on	WO 9740145
JP 2000511407	W	Based on	WO 9740145

PRIORITY APPLN. INFO: US 1996-17785P 19960515; US 1996-16117P 19960424; US 2001-819098 20010411
AN 1997-535830 [49] WPIDS
AB WO 9740145 A UPAB: 19971211
Novel pro-**coagulant** active factor VIII (FVIII), comprises: (a) **human** FVIII (hFVIII) comprising a **mutation** at **Phe309**, preferably **Phe309Ser**; (b) hFVIII comprising the **mutations** Arg336Ile and Arg562Lys; (c) hFVIII comprising a deletion of the B domain and von Willebrand factor binding site, a **mutation** at Arg740 and an addition of an amino acid sequence spacer between the A2 and A3 domains; or (d) hFVIII A1, A2, A3, C1 and C2 domains, which upon thrombin activation becomes a heterodimer comprising an A1 domain fragment and an A2-A3-C1-C2 chain.

USE - The **FVIII protein** can be administered to haemophiliacs, i.e. FVIII replacement therapy, while the nucleic acid molecule can be used for gene therapy.

ADVANTAGE - The FVIII of (a) is capable of recombinant secretion at higher levels than typically obtained with wild type FVIII and retains pro-**coagulant** activity. The FVIII of (b) is resistant to activated protein C (APC) cleavage. The FVIII of (c) and (d) can form a more stable configuration, and have an approximate 5-fold increase in specific activity compared to purified wild type FVIII, while increasing their binding affinity to von Willebrand factor improves their stability.
Dwg.0/16

L18 ANSWER 5 OF 8 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 97450923 MEDLINE
DOCUMENT NUMBER: 97450923 PubMed ID: 9305856
TITLE: Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation factor VIII.
AUTHOR: Swaroop M; Moussalli M; Pipe S W; Kaufman R J
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.
CONTRACT NUMBER: HL52173 (NHLBI)
HL53777 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Sep 26) 272 (39) 24121-4.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB Coagulation factor VIII (FVIII) and factor V are homologous glycoproteins that have a domain structure of A1-A2-B-A3-C1-C2. FVIII is a heterodimer of the heavy chain (domains A1-A2-B) and the light chain (domains A3-C1-C2) in a metal ion-dependent association between the A1- and A3-domains. Previous studies identified a 110-amino acid region within the FVIII A1-domain that inhibits its secretion and contains multiple short peptide sequences that have potential to bind immunoglobulin-binding protein (BiP). FVIII secretion requires high levels of intracellular ATP, consistent with an ATP-dependent release from BiP. Site-directed mutagenesis was used to elucidate the importance of the potential BiP-binding sites in FVIII secretion. Mutation of Phe at position 309 to Ser or Ala enhanced the secretion of functional FVIII and reduced its ATP dependence. The F309S FVIII had a specific activity, thrombin activation profile, and heat inactivation properties similar to those of wild-type FVIII. However, F309S FVIII displayed increased sensitivity to EDTA-mediated inactivation that is known to occur through metal ion chelation-induced dissociation of the heavy and light chains of FVIII. The results support that **Phe309** is important in high affinity heavy and light chain interaction, and this correlates with a high affinity BiP-binding site. Introduction of the F309S mutation into other secretion defective FVIII mutants rescued their secretion, demonstrating the ability of the this mutation to improve secretion of mutant **FVIII proteins** retained in the cell.

L18 ANSWER 6 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97465466 MEDLINE
DOCUMENT NUMBER: 97465466 PubMed ID: 9326186
TITLE: Factor VIII gene analysis in Japanese CRM-positive and CRM-reduced haemophilia A patients by single-strand conformation polymorphism.
AUTHOR: Morichika S; Shima M; Kamisue S; Tanaka I; Imanaka Y; Suzuki H; Shibata H; Pemberton S; Gale K; McVey J; Tuddenham E G; Yoshioka A
CORPORATE SOURCE: Department of Paediatrics, Nara Medical University, Kashihara City, Japan.
SOURCE: BRITISH JOURNAL OF HAEMATOLOGY, (1997 Sep) 98 (4) 901-6.
Journal code: 0372544. ISSN: 0007-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19990129
Entered Medline: 19971209

AB Haemophilia A is the most common X-linked blood **coagulation** disorder; it is caused by deficiency of factor VIII activity (FVIII:C). Half of the affected patients do not have detectable levels of **FVIII protein** in their plasma, whereas about 5% have normal levels of the FVIII antigen (FVIII:Ag) (> 50 u/dl), and are called cross-reacting material (CRM) positive (CRM+ or A+). About 45% of patients have reduced levels of the FVIII:Ag (1-50 u/dl), classified as CRM reduced (CRM[R] or A[R]). We screened the FVIII gene of 13 Japanese patients (five CRM+ and eight CRM[R]) by single-strand conformation polymorphism, and identified 11 different **mutations** in 13 patients by analysing all 26 exons (Trp255Cys, Tyr473Cys, Gly479Arg, Arg531His, Thr667Arg, Arg1689Cys, Arg1941Gln, Arg2150His, Arg2159Cys, Thr2245Ala and Gly2285Val). Seven **mutations** were identified in the A domains (four in the A2 domain). All the **mutations** are point **mutations** resulting in missense codons. Four **mutations** (Trp255Cys, Thr667Arg, Thr2245Ala and Gly2285Val) have not been described previously.

L18 ANSWER 7 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 97384041 MEDLINE
DOCUMENT NUMBER: 97384041 PubMed ID: 9239887
TITLE: [Molecular genetics of hemophilia A].
Genetica molecular de la hemofilia A.
AUTHOR: De Brasi C D; Slavutsky I R; Larripa I B
CORPORATE SOURCE: Departamento de Genetica, Academia Nacional de Medicina, Buenos Aires, Argentina.
SOURCE: MEDICINA, (1996) 56 (5 Pt 1) 509-17. Ref: 32
Journal code: 0204271. ISSN: 0025-7680.
PUB. COUNTRY: Argentina
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Spanish
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19990129
Entered Medline: 19971027

AB Hemophilia A (HemA), an X linked genetic disease, is the most common **coagulation** disorder with an incidence of about 1-2 in 10,000 males and is caused by **mutations** in the factor VIII (FVIII) **coagulation** gene. Firstly, some clinical aspects of the HemA are presented: the current methods to assess both the amount and activity of FVIII, the severity range observed and the presence of inhibitor antibodies against the therapeutic FVIII. Follows a discussion of the relationship of the structural domains of the **FVIII protein** (Figure 1), the aminoacid sequence and their functions. An activation-inactivation model of the successive peptide bonds cleavages of the FVIII is also presented (Figure 2). After the cloning of the FVIII gene in 1984, almost all types of HemA causing **mutations** have been characterized. However, the size and complexity of this gene prevented a screening of the full range of **mutations** for an accurate molecular diagnosis. Moreover, most of the patients with moderate and mild disease have missense **mutations** whereas

approximately half of severe patients have nonsense, frameshift, and some missense **mutations**. There are also less frequently **mutations** such as deletions and insertions leading to severe phenotype and **mutations** affecting mRNA splicing and duplications causing both severe and mild HemA. In order to give genetic counselling in HemA families, studies at the DNA level using intragenic and/ or extragenic polymorphism analysis have been used. But this approach is not entirely satisfactory because it fails in several situations. Most of the causing **mutations** described above are private, and they have been found in only a few unrelated families. Recently, a common molecular inversion of the FVIII gene was identified in 50% of unrelated patients with severe HemA. The copies of a particular DNA sequence (termed F8A gene). One copy is located within intron 22 of the FVIII gene and the other two, 500 kb upstream. An homologous recombination mechanism was proposed for the inversion between an intragenic copy of the F8A gene and either the distal (80% of the inversion) or the proximal copy (20%). Both of these inversions lead to severe HemA because no intact FVIII is produced and can be easily diagnosed by Southern blot analysis. This inversion originates almost exclusively in male germ cells, because pairing Xq with its homologous in female meiosis would probably inhibit the proposed intrachromosome recombination. The molecular analysis of the inversion of intron 22 is now considered as the first line for families with severe HemA patients. In recent years the treatment of patients with hemophilia A and B has been intravenous injection of FVIII or FIX concentrates, respectively. This regimen of regular injection of plasmatic proteins bears a high risk of infection by contaminating viruses (HIV, HBV, etc). Future treatment for patients with hemophilia may include the use of either gene therapy or recombinant **coagulation** factors. Both strategies would completely avoid the infection risk offering a safe and effective treatment for the disease. Recombinant factors, obtained by genetic engineering methods, provide a renewable and unlimited source of FVIII or FIX. The clinical trials of recombinant factors have already started in mid-1995 giving positive results. On the other hand, gene therapy for hemophilia is now in the pre-clinical stage but offers the prospect of a cure for the disease, thus potentially freeing patients from regular injections of the lacking protein. However, experiments in animal models suggest that it may be difficult to obtain adequate therapeutic levels of factors for long periods of time. Recently , a retroviral-mediated gene delivery of **human** FVIII in mice has been reported using the ex vivo strategy of gene therapy. Therapeutic levels of FVIII in the circulation were obtained for > 1 week and it was also observed that the capacity of primary cells to deliver FVIII in blood was strongly dependent on

L18 ANSWER 8 OF 8 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 90001543 MEDLINE
DOCUMENT NUMBER: 90001543 PubMed ID: 2506948
TITLE: An arginine to cysteine amino acid substitution at a critical thrombin cleavage site in a dysfunctional factor VIII molecule.
AUTHOR: Shima M; Ware J; Yoshioka A; Fukui H; Fulcher C A
CORPORATE SOURCE: Scripps Clinic and Research Foundation, La Jolla, CA.
CONTRACT NUMBER: HL 31950 (NHLBI)
SOURCE: BLOOD, (1989 Oct) 74 (5) 1612-7.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198911

ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 20000303
Entered Medline: 19891101

AB We have analyzed the factor VIII (**FVIII**) **protein** and the nucleotide sequence around two thrombin cleavage sites, at arginine 372 in the FVIII heavy chain and arginine 1689 in the FVIII light chain in a naturally occurring dysfunctional FVIII variant, FVIII Okayama. The patient was a 42-year-old hemophiliac with a FVIII **coagulant** activity of 0.03 U/mL and a FVIII antigen level of 0.8 U/mL. The patient's FVIII was not thrombin activatable to levels seen in normal plasma. Immunoblotting of partially purified FVIII Okayama and normal FVIII showed that thrombin cleavage of the 92 kilodalton (Kd) heavy chain was impaired in the mutant protein. The patient's genomic DNA was amplified using the polymerase chain reaction with two sets of synthetic oligonucleotide primers spanning amino acid residues 319 to 400 and 1630 to 1720. Sequence analysis of the amplified DNA fragments revealed a cytosine to thymine transition, converting an arginine to a cysteine codon at residue 372. No abnormality was found in the FVIII light chain region analyzed. The patient's hemophilic brother and carrier mother revealed the same **mutation**. We conclude that the pathogenesis of hemophilia A in this patient is probably due to an arginine to cysteine substitution at a thrombin cleavage site in the FVIII heavy chain.